AMENDMENTS

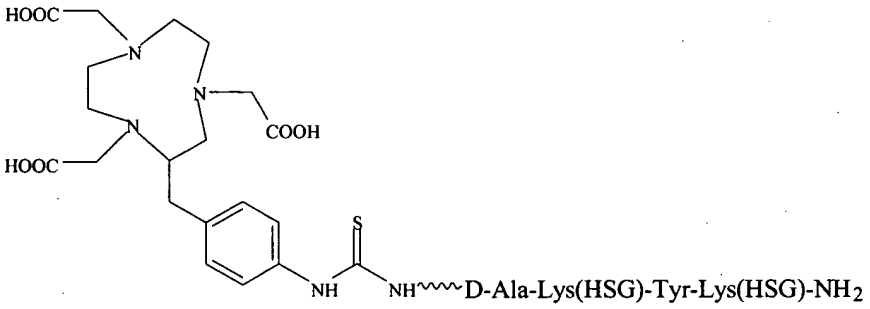
Please delete the paragraph on page 3, lines 6-12, and replace it with the following amended paragraph:

Further, the invention provides pre-targeting methods of diagnosis and therapy using the combination of bi-specific antibody and the targetable conjugates:

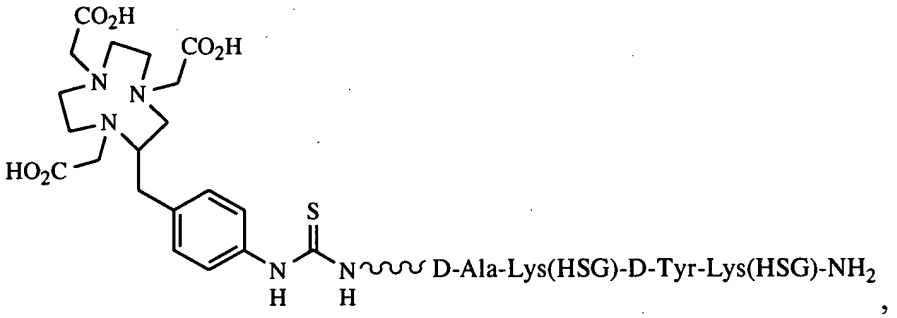
(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;

(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2; **(SEQ ID NO: 15)**

(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;

(d) 

; and

(e) 

,

as well as methods of making the bi-specifics, and kits for use in such methods.

Please delete the paragraphs bridging page 4-12, and replace them with the following amended paragraph:

The invention further relates to a method for detecting or treating target cells, tissues or pathogens in a mammal, comprising:

administering an effective amount of a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate;

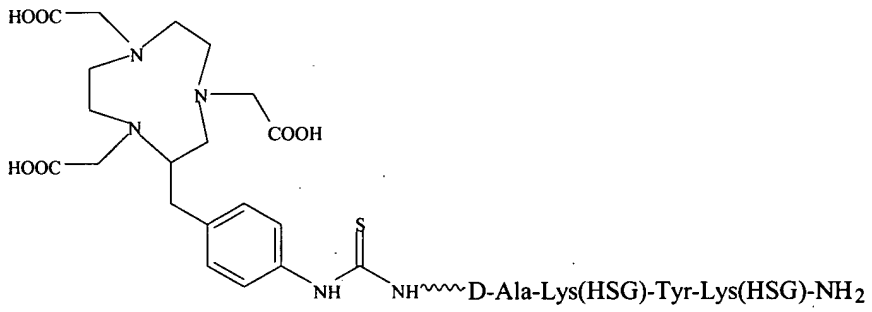
wherein said at least one arm is capable of binding to a complementary binding moiety on the target cells, tissues or pathogen or on a molecule produced by or associated therewith; and

administering a targetable conjugate selected from the group consisting of

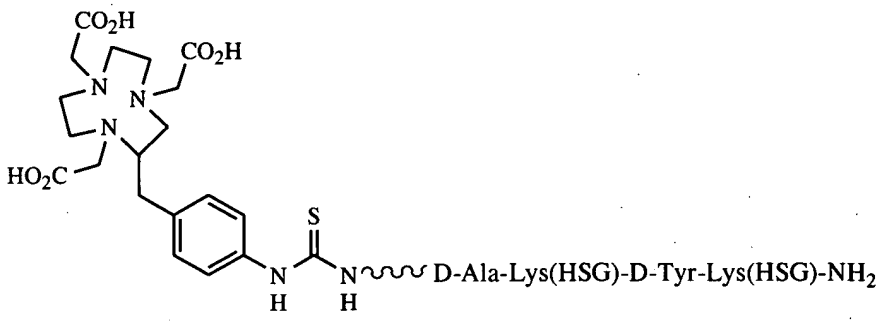
(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;

(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2; **(SEQ ID NO: 15)**

(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;

(d) 

; and

(e) 

The invention further relates to a method of treating or identifying diseased tissues in a subject, comprising:

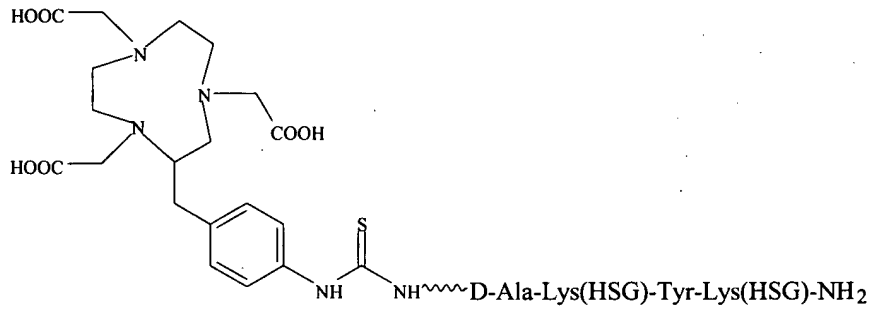
administering to said subject a bi-specific antibody or antibody fragment having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate;

optionally, administering to said subject a clearing composition, and allowing said composition to clear non-localized antibodies or antibody fragments from circulation; and administering to said subject a targetable conjugate selected from the group consisting of:

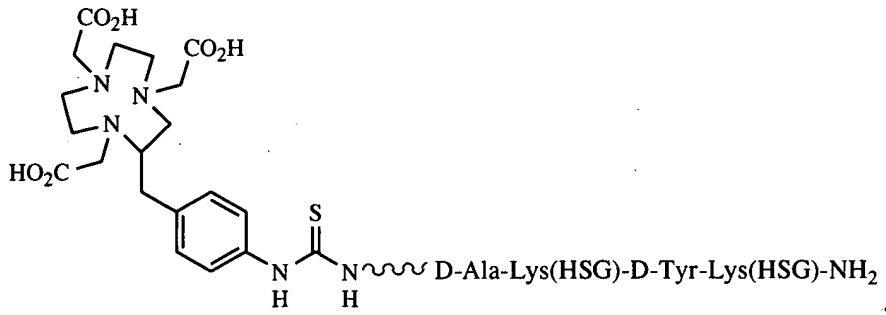
(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;

(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2; **(SEQ ID NO: 15)**

(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;

(d) 

; and

(e) 

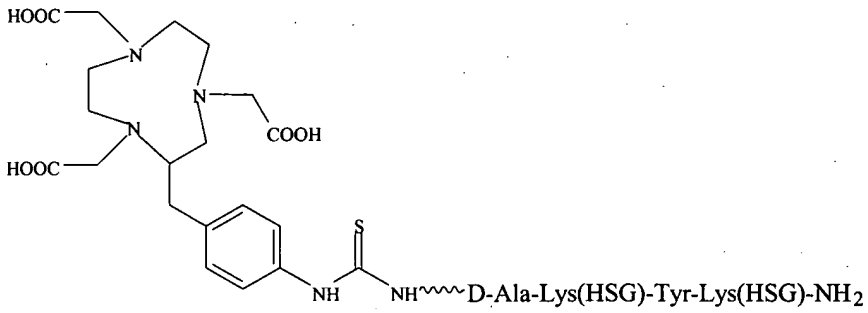
The invention further relates to a kit useful for treating or identifying diseased tissues in a subject comprising:

(A) a bi-specific antibody or antibody fragment having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate, wherein said conjugate is selected from the group consisting of

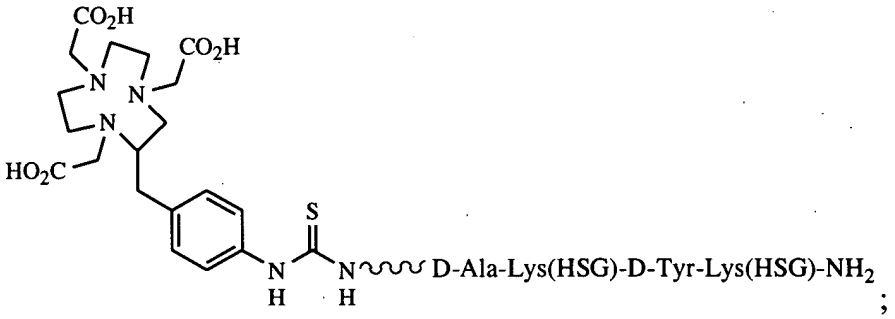
(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;

(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2; **(SEQ ID NO: 15)**

(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;

(d) 

; and

(e) 

;

(B) a targetable conjugate which comprises a carrier portion which comprises or bears at least one epitope recognizable by said at least one other arm of said bi-specific antibody or antibody fragment, and one or more conjugated therapeutic or diagnostic agents, or enzymes; and

(C) optionally, a clearing composition useful for clearing non-localized antibodies and antibody fragments; and

(D) optionally, when said first targetable conjugate comprises an enzyme,

1) a prodrug, when said enzyme is capable of converting said prodrug to a drug at the target site; or

2) a drug which is capable of being detoxified in said subject to form an intermediate of lower toxicity, when said enzyme is capable of reconverting said detoxified intermediate to a toxic form, and, therefore, of increasing the toxicity of said drug at the target site, or

3) a prodrug which is activated in said subject through

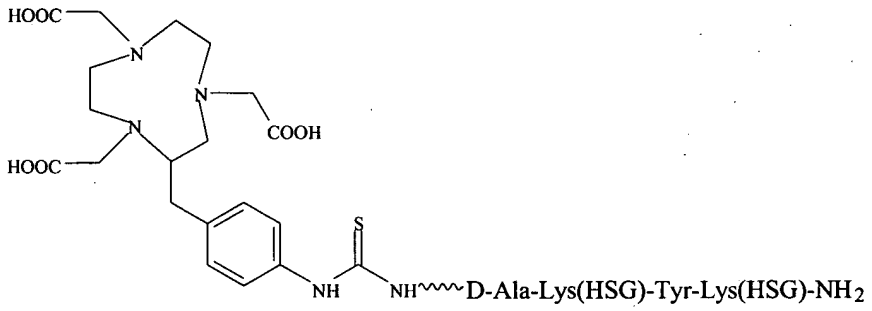
natural processes and is subject to detoxification by conversion to an intermediate of lower toxicity, when said enzyme is capable of reconverting said detoxified intermediate to a toxic form, and, therefore, of increasing the toxicity of said drug at the target site.

The invention further relates to a targetable conjugate selected from the group consisting of:

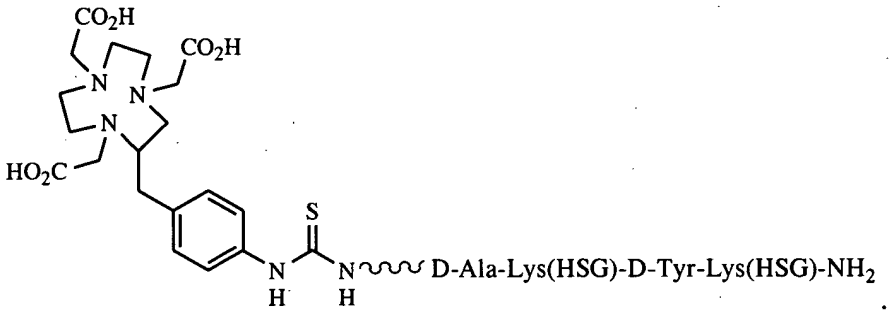
(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;

(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2; **(SEQ ID NO: 15)**

(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;

(d) 

; and

(e) 

The invention further relates to a method of screening for a targetable conjugate comprising:

contacting said targetable construct with a bi-specific antibody or antibody fragment having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds said targetable conjugate to give a mixture;

wherein said at least one arm is capable of binding to a complementary binding moiety on the target cells, tissues or pathogen or on a molecule produced by or associated therewith; and

optionally incubating said mixture; and

analyzing said mixture.

The invention further relates to a method for imaging normal tissue in a mammal, comprising:

administering an effective amount of a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate;

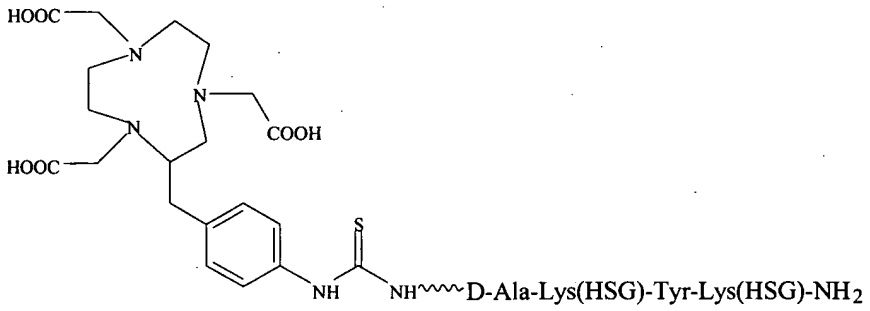
wherein said at least one arm is capable of binding to a complementary binding moiety on the target cells, tissues or pathogen or on a molecule produced by or associated therewith; and

administering a targetable conjugate selected from the group consisting of

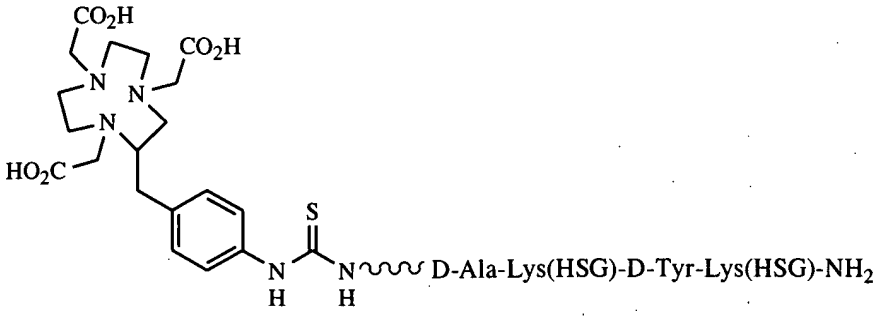
(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;

(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2; **(SEQ ID NO: 15)**

(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;

(d) 

; and

(e) 

The invention further relates to a method of intraoperatively identifying diseased tissues, in a subject, comprising:

administering an effective amount of a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate;

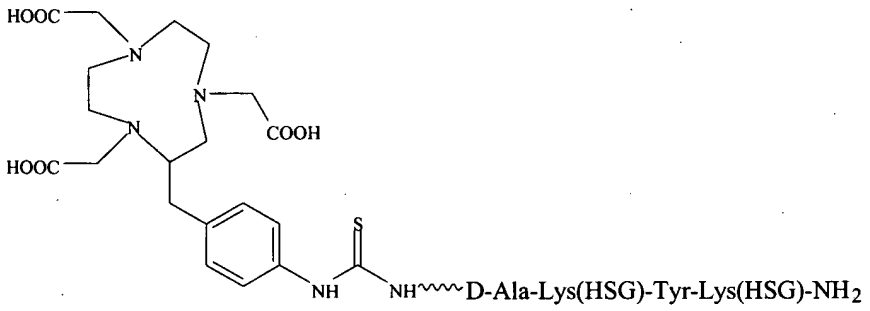
wherein said at least one arm is capable of binding to a complementary binding moiety on the target cells, tissues or pathogen or on a molecule produced by or associated therewith; and

administering a targetable conjugate selected from the group consisting of

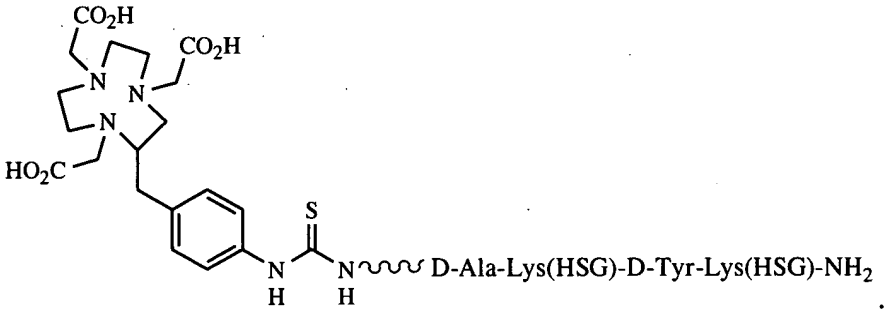
(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;

(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2; **(SEQ ID NO: 15)**

(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;

(d) 

; and

(e) 

The invention further relates to a method for the endoscopic identification of diseased tissues, in a subject, comprising:

administering an effective amount of a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate;

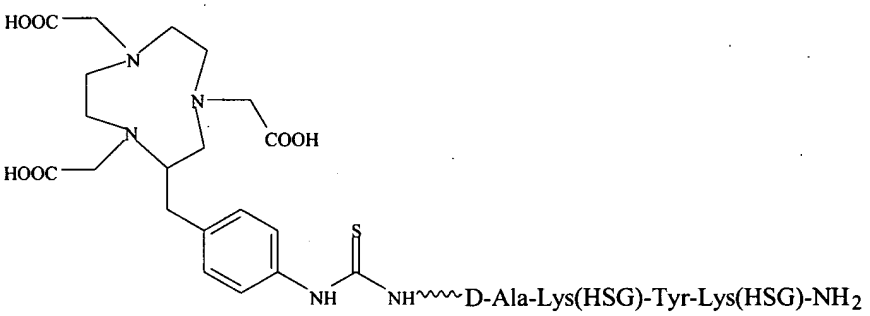
wherein said at least one arm is capable of binding to a complementary binding moiety on the target cells, tissues or pathogen or on a molecule produced by or associated therewith; and

administering a targetable conjugate selected from the group consisting of

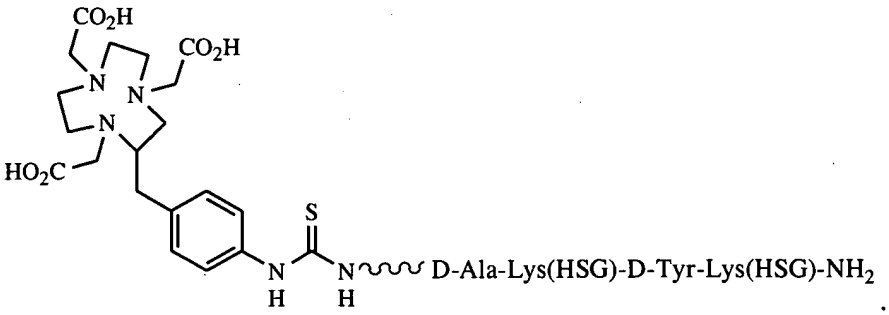
(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;

(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2; **(SEQ ID NO: 15)**

(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;

(d) 

; and

(e) 

The intenvtion further relates to a method for the intravascular identification of diseased tissues, in a subject, comprising:

administering an effective amount of a bispecific antibody or antibody fragment comprising at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate;

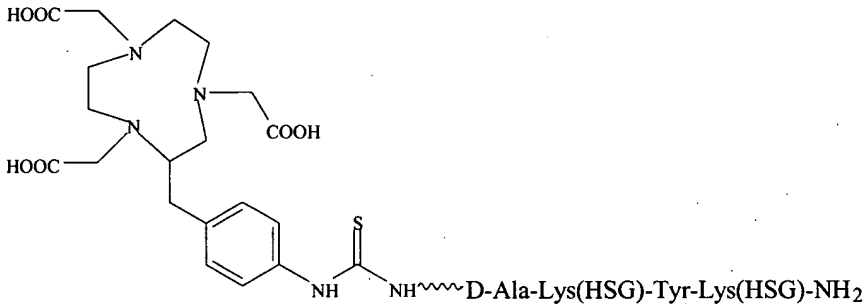
wherein said at least one arm is capable of binding to a complementary binding moiety on the target cells, tissues or pathogen or on a molecule produced by or associated therewith; and

administering a targetable conjugate selected from the group consisting of

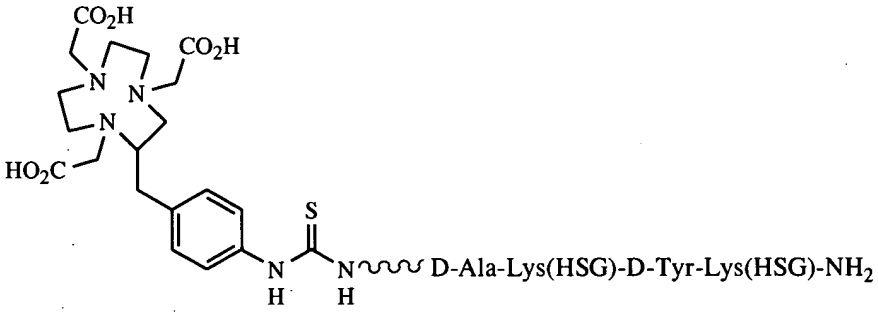
(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;

(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2; **(SEQ ID NO: 15)**

(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;

(d) 

; and

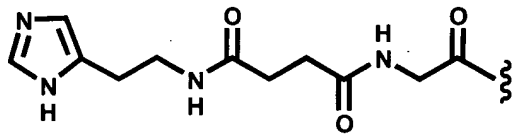
(e) 

Brief Description of the Drawings

FIGS. 1-7 show the polypeptide sequence for MAb 679 V_k **(SEQ ID NOS 1-2)**, MAb 679 V_h **(SEQ ID NOS 3-4)**, MAb 679 scFv **(SEQ ID NOS 5-6)**, Mu9 V_k **(SEQ ID NOS 7-8)**, Mu9 V_h **(SEQ ID NOS 9-10)**, hMu9 V_k **(SEQ ID NOS 11-12)**, and hMu9 V_h **(SEQ ID NOS 13-14)**, respectively. The corresponding polynucleotide which codes for said polypeptides are also shown in Figures 1-7.

Please delete the paragraph bridging pages 14-15, and replace it with the following amended paragraph:

Peptides having as few as two amino-acid residues may be used, preferably two to ten residues, if also coupled to other moieties such as chelating agents. The linker should be a low molecular weight conjugate, preferably having a molecular weight of less than 50,000 daltons, and advantageously less than about 20,000 daltons, 10,000 daltons or 5,000 daltons, including the metal ions in the chelates. For instance, the known peptide DTPA-Tyr-Lys(DTPA)-OH (wherein DTPA is diethylenetriaminepentaacetic acid) has been used to generate antibodies against the indium-DTPA portion of the molecule. However, by use of the non-indium-containing molecule, and appropriate screening steps, new Abs against the tyrosyl-lysine dipeptide can be made. More usually, the antigenic peptide will have four or more residues, such as the peptide DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2 **(SEQ ID NO: 15)**, wherein DOTA is 1,4,7,10-tetraazacyclododecanetetraacetic acid and HSG is the histamine succinyl glycyl group of the formula:



The non-metal-containing peptide may be used as an immunogen, with resultant Abs screened for reactivity against the Phe-Lys-Tyr-Lys **(SEQ ID NO: 18)** backbone.

Please delete the paragraph on page 17, lines 4-20, and replace it with the following amended paragraph:

Chelators such as those disclosed in U.S. Patent 5,753,206, especially thiosemi-carbazonylglyoxylcysteine(Tscg-Cys) and thiosemicarbazinyl-acetylcysteine (Tsca-Cys) chelators are advantageously used to bind soft acid cations of Tc, Re, Bi and other transition metals, lanthanides and actinides that are tightly bound to soft base ligands, especially sulfur- or

phosphorus-containing ligands. It can be useful to link more than one type of chelator to a peptide, e.g., a DTPA or similar chelator for, say In(III) cations, and a thiol-containing chelator, e.g., Tscg-Cys, for Tc cations. Because antibodies to a di-DTPA hapten are known (Barbet '395, *supra*) and are readily coupled to a targeting antibody to form a bsAb, it is possible to use a peptide hapten with cold diDTPA chelator and another chelator for binding a radioisotope, in a pretargeting protocol, for targeting the radioisotope. One example of such a peptide is Ac-Lys(DTPA)-Tyr-Lys(DTPA)-Lys(Tscg-Cys-)-NH_2 **(SEQ ID NO: 16)**. This peptide can be preloaded with In(III) and then labeled with 99-m-Tc cations, the In(III) ions being preferentially chelated by the DTPA and the Tc cations binding preferentially to the thiol-containing Tscg-Cys. Other hard acid chelators such as NOTA, DOTA, TETA and the like can be substituted for the DTPA groups, and Mabs specific to them can be produced using analogous techniques to those used to generate the anti-di-DTPA Mab.

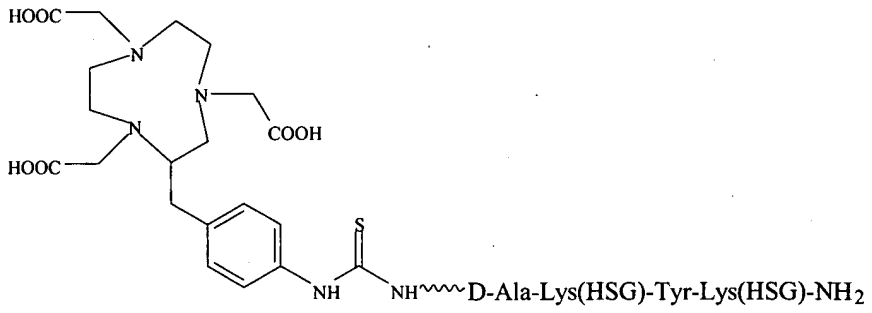
Please delete the paragraph bridging pages 17-18 and replace it with the following amended paragraph:

Preferred chelators include NOTA, DOTA and Tscg and combinations thereof. These chelators have been incorporated into a chelator-peptide conjugate motif as exemplified in the following constructs:

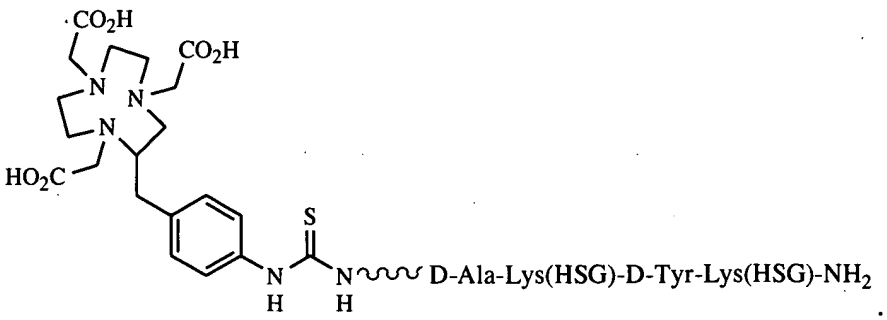
(a) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2;

(b) DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2; **(SEQ ID NO: 15)**

(c) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2;

(d) 

; and

(e) 

Please delete the paragraph bridging pages 18-19 and replace it with the following amended paragraph:

Chelators are coupled to the linker moieties using standard chemistries which are discussed more fully in the working Examples below. Briefly, the synthesis of the peptide Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys-)-NH_2 was accomplished by first attaching Aloc-Lys(Fmoc)-OH to a Rink amide resin on the peptide synthesizer. The protecting group abbreviations "Aloc" and "Fmoc" used herein refer to the groups allyloxycarbonyl and fluorenylmethyloxy carbonyl. The Fmoc-Cys(Trt)-OH and TscG were then added to the side chain of the lysine using standard Fmoc automated synthesis protocols to form the following peptide: Aloc-Lys(Tscg-Cys(Trt)-rink resin. The Aloc group was then removed. The peptide synthesis was then continued on the synthesizer to make the following peptide: (Lys(Aloc)-D-Tyr-Lys(Aloc)-Lys(Tscg-Cys(Trt)-)-rink resin. Following N-terminus acylation, and removal of the side chain Aloc protecting groups. The resulting peptide was then treated with activated N-trityl-HSG-OH until the resin gave a negative test for amines using the Kaiser test. *See* Karacay *et al. Bioconjugate Chem. 11*:842-854 (2000). The synthesis of Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys-)-NH_2, as well as the syntheses of DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2; and DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2 **(SEQ ID NO: 15)** are described in greater detail below.

Please delete the paragraph bridging pages 31-32 and replace it with the following amended paragraph:

Functional bi-specific single-chain antibodies (bscAb), also called diabodies, can be produced in mammalian cells using recombinant methods. See, e.g., Mack *et al.*, *Proc. Natl. Acad. Sci.*, 92: 7021-7025, 1995. For example, bscAb are produced by joining two single-chain Fv fragments via a glycine-serine linker using recombinant methods. The V light-chain (V_L) and V heavy-chain (V_H) domains of two antibodies of interest are isolated using standard PCR methods. The V_L and V_H cDNA's obtained from each hybridoma are then joined to form a single-chain fragment in a two-step fusion PCR. The first PCR step introduces the $(Gly_4\text{-}Ser_1)_3$ **(SEQ ID NO: 17)** linker, and the second step joins the V_L and V_H amplicons. Each single chain molecule is then cloned into a bacterial expression vector. Following amplification, one of the single-chain molecules is excised and sub-cloned into the other vector, containing the second single-chain molecule of interest. The resulting bscAb fragment is subcloned into an eukaryotic expression vector. Functional protein expression can be obtained by transfecting the vector into chinese hamster ovary cells. Bi-specific fusion proteins are prepared in a similar manner. Bi-specific single-chain antibodies and bi-specific fusion proteins are included within the scope of the present invention.

Please delete the paragraph bridging pages 36-37 and replace it with the following amended paragraph:

C. Peptides for Carrying Therapeutic/Imaging Radioisotopes to Tumors via Bispecific Antibody Tumor Pretargeting

DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2 **(SEQ ID NO: 15)** (IMP 237) was synthesized to deliver therapeutic radioisotopes such as ^{90}Y or ^{177}Lu to tumors via bispecific antibody tumor pretargeting. The bispecific antibody is composed of one portion which binds to an antigen on the tumor and another portion which binds to the HSG peptide. The antibody which binds the HSG peptide is 679. This system can also be used to deliver imaging isotopes such as ^{111}In-111.

Please delete the paragraph bridging pages 38-39 and replace it with the following amended paragraph:

Radiolabeling

^{90}Y Kit Preparation

DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH_2 **(SEQ ID NO: 15)** was dissolved in 0.25 M NH_4OAc/ 10 % HPCD buffer at concentrations of 9, 18, 35, 70 and 140 μg/mL. The solutions were sterile filtered through a 0.22 μm Millex GV filter in one mL aliquots into acid washed lyophilization vials. The filled vials were frozen immediately on filling and lyophilized. When the lyophilization cycle was complete the vials were sealed under vacuum and crimp sealed upon removal from the lyophilizer.

Please delete the paragraph on page 41, lines 1-2, and replace it with the following amended paragraph:

Serum Stability of DOTA-Phe-Lys(HSG)-Tyr-Lys(HSG)-NH2* (SEQ ID NO: 15) *(IMP 237) and DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH2 (IMP 241)